

Enzymatic studies in the liver and muscle of freshwater fish, *Pangasius hypophthalmus* exposed to tannery effluent

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ABSTRACT:

The effects of effluents from tanning industry and its toxicity on enzymatic changes of a fresh water fish, *Pangasius hypophthalmus* were studied. Tannery effluent was diluted with normal water at two different concentration (1ppm, 2ppm) to which fishes were exposed for 10 days. The diluted tannery effluent was analyzed for the presence of heavy metals. Heavy metals like cadmium, chromium, lead, zinc, etc., were present in the tannery effluent diluted water. Fishes were dissected on the 5th and 10th day. Liver and muscle tissues were removed for enzymatic analysis. The levels of the enzymes (Aspartate Transaminase, Alanine Transaminase, Catalase, Superoxide Dismutase, Reduced Glutathione, Glutathione-S-Transferase and Glutathione Peroxidase) were decreased in the Liver, whereas there was an increased activity of the enzymes in the muscle. This investigation shows that fishes are affected by tannery effluent even at very low dilutions. These changes can affect human life through food web. Hence the tannery effluent must be treated and then discharged into the water sources as a safety measure to aquatic and human life. The observations made in the investigations interpret that fishes also shows an adaptive measure to protect themselves from the toxic effect of the tannery effluent.

KEY WORDS: effluent; tannery; *Pangasius hypophthalmus*; enzymes

INTRODUCTION

The birth of Leather in India dates back to 3,000 years B.C. Indian Leather industry is the 6th largest in the world and is one of the major established manufacturing industries in the modern as well as traditional sector. The pollution load from the tanning activity has been estimated to be 50% more in weight than the weight of the hides processed. Pollution comes from several of the sub-processes due to the usage of 175 different chemicals and is both organic and chemical [1]. Wastes and chemicals released to water system are the main pollution concerns for the leather industry. They are produced during washing, dehairing and tanning of the leather. The water discharge from the turnover is called Raw Effluent.

The damage to the environment by the hazardous tannery effluent is becoming an acute problem in the country. Tannery wastewaters are mainly characterized by high salinity, high organic loading and specific pollutants such as chromium, strong color (reddish dull brown), high BOD, high pH, high dissolved solids etc [2]. Comparing the water bodies, ground water is normally preferred for drinking and human usage because it tends to be less contaminated directly by wastes and organisms [3]. The untreated sewage, industrial effluents and agricultural wastes are often discharged into the water bodies. The contamination and pollution of water bodies spread a wide range of water borne diseases [4-5]. Due to the impact of effluents, the ground water in and around the leather industry has become unfit for drinking and irrigation [6]. Plants and aquatic organisms growing in such water bodies may absorb heavy metals into their physiological system directly or indirectly. The concentration and composition of industrial effluents

brings physiological and behavioral changes in the fishes living in the environment. When the living animals are exposed to polluted water it ceases to damage the internal organs too. Accumulation of the heavy metals at various levels of animals in the food chain will finally reach man and hence is of great importance in the ecological cycle [7]. The present study investigated the impact of tannery effluent on the enzymatic activity of the muscle and liver of the fresh water fish, *Pangasius hypophthalmus*.

MATERIALS AND METHODS:

Tannery Effluent:

The tannery effluent was collected directly from a tannery industry in Pallavaram (Chennai, Tamil Nadu). The color of the effluent was brown. It had a bad odour and a pungent smell. Tannery effluent contained many dissolved particles. The tannery effluent was diluted with deionised water at two different concentrations (1ppm, 2ppm) for the experimental work. Tanks were filled with diluted tannery effluent to which fishes were added and treated for 10 days.

Experimental Fishes:

The *Pangasius hypophthalmus* were bought from local lake near kolathur, Chennai. It was acclimatized for 10 days in normal tap water. It was placed in glass tanks of length 5feet, breadth 1.5feet and height 3feet. During the period of acclimatization the fishes were fed once in a day at morning by normal aquarium flake food.

Biochemical analysis:

Healthy fishes showing active movements were only taken for the study. Six fishes from each tank were dissected on the 5th and 10th day. Liver and muscle

tissues were removed, homogenated with 5 ml saline and stored in refrigerator for further enzyme analysis.

Protein contents in the Liver and Muscle were determined colorimetrically [8] using Bovine serum albumin as standard.

Activities of aspartate transaminase (ALT) and alanine transaminase (AST) were determined colorimetrically [9].

Catalase (CAT) activity was estimated by following the absorbance of hydrogen peroxide at 570 nm [10]. Superoxide dismutase (SOD) activity was estimated by measuring the inhibition of autoxidation of epinephrine at pH 10.2 [11]. Reduced glutathione (GSH) was estimated in the liver and muscle homogenates using 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) [12]. Glutathione-S-Transferase (GST) activity using 1 chloro 2,4 dinitrobenzene as substrate [13]. Glutathione Peroxidase (GPx) was assayed according to the method of Rotruck *et al.*, [14].

TABLE NO 1: The activities of the Enzymes AST and ALT, (μmol of CDNB conjugated / min / mg ptn) CAT (μmol of H_2O_2 / min / mg ptn) and SOD (μmol of units/ min / mg of ptn.) in the Liver and Muscle of fresh water fish *pangasius hypophthalmus* treated with tannery effluent for 5 and 10 days:

ENZYMES		TISSUE	CONTROL	EXPERIMENTAL GROUP		F VALUE	P VALUE
				1ppm	2ppm		
AST	5 TH DAY	Liver	0.662±0.020	0.205±0.064	0.231±0.170	101.7	P<0.0001
		Muscle	0.157±0	0.380±0.137	0.765±0.242	27.65	P<0.0001
	10 TH DAY	Liver	0.66±0.20	0.200±0.056	0.256±0.092	101.7	P<0.0001
		Muscle	0.157±0	0.186±0.087	0.276±0.060	27.65	P<0.0001
ALT	5 TH DAY	Liver	0.248±0.071	0.028±0.009	0.082±0.021	81.49	P<0.0001
		Muscle	0.096±0.063	0.106±0.036	0.160±0.073	1.269	0.2957
	10 TH DAY	Liver	0.248±0.071	0.046±0.033	0.0262±0.013	81.49	P<0.0001
		Muscle	0.096±0.063	0.070±0.010	0.090±0.081	1.269	0.2957
CAT	5 TH DAY	Liver	32.43±29.42	10.24±6.26	5.98±2.87	2.987	0.0656*
		Muscle	70.1±51.4	96.97±51.58	243.48±52.58	10.41	P<0.0001
	10 TH DAY	Liver	32.43±29.42	19.59±8.35	27.46±19.67	2.987	0.0656*
		Muscle	70.1±51.4	24.29±21.10	29.50±24.41	10.41	P<0.0001
SOD	5 TH DAY	Liver	0.711±1.15	0.168±0.272	0.124 ±0.189	2.986	0.0657*
		Muscle	0.28 ±0.327	0.818±1.352	0.46±0.727	2.07	0.1439
	10 TH DAY	Liver	0.711±1.15	0.009±0.006	0.197±0.202	2.986	0.0657*
		Muscle	0.285±0.327	1.212±2.007	0.0159±0.015	2.07	0.1439

TABLE NO 2: The activities of the Enzymes GSH (μmol of μg of GSH / g wet tissue), GST (μmol of CDNB conjugated / min / mg ptn), GPx (μmol of μg / min / mg ptn) in the Liver and Muscle of fresh water fish *pangasius hypophthalmus* treated with tannery effluent for 5 and 10 days:

ENZYMES		TISSUE	CONTROL	EXPERIMENTAL GROUP		F VALUE	P VALUE
				1ppm	2ppm		
GSH	5 TH DAY	Liver	292.3±74.13	199.13±64.00	225.43±26.78	22.79	0.0001**
		Muscle	191.5±37.72	217.76±14.83	192.73±15.77	1.936	0.1794
	10 TH DAY	Liver	292.3±74.13	144.26±15.0	208.63±20.05	22.79	0.0001**
		Muscle	191.5±37.72	200.33±27.43	218.33±16.51	1.936	0.1794
GST	5 TH DAY	Liver	1.462±1.165	1.038±0.971	0.433 ±0.120	3.842	0.0327*
		Muscle	0.67±0.503	1.648±1.481	1.445±0.488	1.395	0.2634
	10 TH DAY	Liver	1.462±1.165	0.324±0.046	0.875±0.522	3.842	0.0327*
		Muscle	0.66±0.503	0.514±0.256	0.765±0.292	1.395	0.2634
GPx	5 TH DAY	Liver	2.368±0.344	0.146 ±0.094	0.290±0.035	399.5	P<0.0001
		Muscle	1.276±0.643	0.588±0.183	1.494±0.535	10.74	0.0003
	10 TH DAY	Liver	2.368±0.344	0.151±0.175	0.246±0.048	399.5	P<0.0001
		Muscle	1.276±0.643	0.261±0.216	0.756±0.435	10.74	0.0003

Values are Mean ± SD (n = 6)

P < 0.0001 → Signify appreciable difference

P Value 0.01 to 0.05 → * denotes significance at 5% level

P Value < 0.01 → ** denotes significance at 1% level (highly significant)

RESULTS AND DISCUSSION:

ACTIVITY OF ASPARTATE AND ALANINE TRANSAMINASES IN THE EXPERIMENTAL FISHES:

The activity of AST (Aspartate transaminase) decreased in the liver and increased in the muscle of the test group exposed to tannery effluent (1ppm and 2ppm) and it is depicted in the Table no 1. The difference in activity of AST in liver of experimental fish is shown in Figure no 1 and in muscle is shown in Figure no 2.

The activity of ALT (Alanine Transaminase) is decreased in the liver of the test group exposed to tannery effluent (1ppm and 2ppm) as shown in the Table no 1. An increasing trend in the activity of ALT in the muscles were observed in the fishes exposed to tannery effluent for 5 and 10 days which is depicted in the Figure no 3. Whereas there was a decreased activity of ALT in the muscle of test group exposed to 2ppm of tannery effluent for 10 days. The result of ALT assay in liver and muscle of the experimental fishes is shown in Figure no 3 and 4.

Our results showed similar findings where, the effect of arsenic in the carp, *Labeo rohita* decreased the activity of AST and ALT [15]. Significantly decreased activity of ALT and AST activity was observed in the liver of *Clarias gariepinus* [16], whereas there was an increased activity in the muscle of catfish when exposed to aqueous extracts of *Lapidagathis alopecuroides* leaves [17]. The activities of transaminases (AST and ALT) increased in muscle of *Brycon cephalus* when exposed to phenol [18].

Since ALT and AST function as a link between carbohydrate and protein metabolism by catalyzing the inter conversion of strategic compounds like α -keto glutarate and alanine to pyruvic acid and glutamic acid respectively (Kreb's cycle) and in the process of meeting the energy demand of the organs in crisis [19]. Thus decreased activity of AST and ALT in the liver indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system. Such damage to cell organelles were reported in various studies [20-21]. Whereas the increased level of muscle AST and ALT

ACTIVITY OF SUPEROXIDE DISMUTASE IN THE EXPERIMENTAL FISHES:

The activity of superoxide dismutase in the liver was found to be decreased in all the test group of fishes exposed to tannery effluent for different duration of days which is shown in Table no 1. Figure no 7 and 8 and indicates the difference in the activity of SOD in

activities was a result of the disturbances in the Kreb's cycle [22].

ACTIVITY OF CATALASE IN THE EXPERIMENTAL FISHES:

Catalase activity in the liver tissue was found to be decreased in all the group of fishes exposed to tannery effluent which is shown in the Table no 1. Figure no 5 and 6 indicates the activity of catalase in liver and muscle tissues, respectively, in experimental group of fishes exposed for different duration.

In the muscle tissue shown in Table no 1, there is an increased and decreased activity of catalase in group of fish exposed to tannery effluent for 5 and 10 days respectively.

The observed results correlates with the previous investigations performed on different freshwater fish with heavy metals and organic pollutants. The CAT activity was significantly decreased in the liver of *Heteropneustes fossilis* [23]. The catalase activity was reduced in liver with increasing duration of arsenic exposure in fishes [24]. The exposure of 0.4mg/L of copper decreased CAT activity in liver of *Piaractus mesopotamicus* [25]

The activity of catalase was significantly reduced in the liver and muscle of *Clarias gariepinus* from the Ogun River contaminated with heavy metals [26]. The catalase activity at the contaminated stations, Hasiveilar and Boran was found to be lower than at the relatively uncontaminated station, Imikusagi [27].

A sharp decrease in catalase activity was observed in *Oreochromis niloticus* exposed to silver [28]. The CAT activity of Liver was found to be inhibited following both in vivo and in vitro exposure to dissolved cadmium at a concentration greater than 1mg/l in the killifish, *Fundulus heteroclitus* and it is suggested that there is a direct effect of cadmium on high molecular weight compounds like catalase [29].

The decreased catalase activity may be due to the flux of superoxide radicals which have been shown to inhibit CAT activity. This decreased level indicates a reduced ability to protect cells against hydrogen peroxide [30].

liver and muscle tissues, respectively, in group of fishes exposed for different duration of days.

An increasing trend in the activity of SOD in the muscle were observed in the group of fishes exposed to tannery effluent (1ppm and 2ppm) for 5 and 10 days which is shown in the Table 1. Whereas there was a decreased activity of SOD in the muscle of the test

group exposed to 2ppm of tannery effluent for 10 days depicted in Figure no 8.

The findings of the present study were in accordance with investigations performed on different freshwater fishes with different pollutants. The activity of SOD were decreased in the liver tissues of Tilapia fish when exposed to microcystins that induced oxidative stress [31]. The exposure of Cadmium in *Oreochromis niloticus* altered the activity of SOD in the liver and muscle tissues [32].

SOD converts superoxide anion (Oxygen) to hydrogen peroxide. The increased SOD activity in tissues indicates that more protein is required to protect the cells against superoxide radicals [27]. Thus the decreased SOD activity might be due to the lack of protein for cell protection.

ACTIVITY OF REDUCED GLUTATHIONE IN THE EXPERIMENTAL FISHES:

In the present study, the reduced glutathione in liver exhibited a decreasing trend whereas, the muscle showed an increasing trend in the group of fishes exposed to different concentration of tannery effluent for different duration of days which is shown in Figure no: 9 and 10. Table no 2 indicates the content of reduced glutathione in liver and muscle in the experimental group of fishes exposed for different duration.

The findings of this study were in accordance with investigations performed on different freshwater fishes with different pollutants. Reduced glutathione levels depleted progressively in liver of Nile tilapia (*Oreochromis niloticus*) exposed to cadmium [33].

GSH content was significantly decreased in the liver of *Cyprinus carpio* due to high concentrations of hexachlorobenzene [34]. Decrease in glutathione content was observed in the liver of freshwater teleost, *Ictalurus melas* [35]. Decreased GSH content was observed in liver of *Channa punctatus* after exposure to mercury for 30 days [36].

The apparent increase in GSH levels in the muscle with concomitant elevation in the activity of GST in the organs suggests an adaptive measure of the fishes and confirms the protective role of this biomolecule against oxidative stress induced by the heavy metals [37].

ACTIVITY OF GLUTATHIONE-S-TRANSFERASE IN THE EXPERIMENTAL FISHES:

GST activity was decreased in liver tissues and increased in muscle tissues of the test group exposed to

the tannery effluent which is shown in Table: 2. Figure: 11 and 12 indicates the difference in the activity of GST in liver and muscle tissues, respectively, in test and control groups.

Similar results were observed in the previous investigations reported by many authors. Increase in GST activity was found in muscles of gold fish *Carassius auratus* during anoxia and oxidative stress [38].

Our results showed similar findings as that of P.Kavitha and J.Venkateswara Rao who reported that GST activity showed transient increase in muscle of euryhaline fish *Oreochromis mossambicus* exposed to sub-lethal concentration of profenofos [39]. Significantly increased GST activity was observed in the muscle of tilapia, *Oreochromis niloticus* exposed to Total ammonia nitrogen [40].

ACTIVITY OF GLUTATHIONE PEROXIDASE IN THE EXPERIMENTAL FISHES:

The activity of Glutathione peroxidase in the liver tissue was found to be decreased in the test group exposed to different duration of days depicted in the Figure no: 13. The activity of GPx is significantly increased in the muscle when exposed to tannery effluent (1ppm and 2ppm) for 5 days and decreased activity is observed in the muscle tissue exposed to tannery effluent for 10 days which is shown in the Figure no: 14.

Our results showed similar findings as that of Pandey S *et al.*, who reported that the activity of GPx is significantly decreased in the liver of the fish *Channa punctatus* [41].

A decreased activity of GPx was observed in the liver of the fish *Carassius auratus* exposed to water with zinc accumulation [42]. The effects of the dithiocarbamate Thiram decreased the activity of glutathione peroxidase in the liver tissue of *Rainbow trout* [43]. Glutathione peroxidase activity was increased in the muscle of fish dosed with Tetrachlorobiphenyl [44].

Our study indicates that the glutathione system constitutes a sensitive biochemical indicator of chemical pollution. The toxins present in the diluted effluent affect the levels of glutathione and glutathione-dependent enzymes in liver and muscle of the experimental fishes.

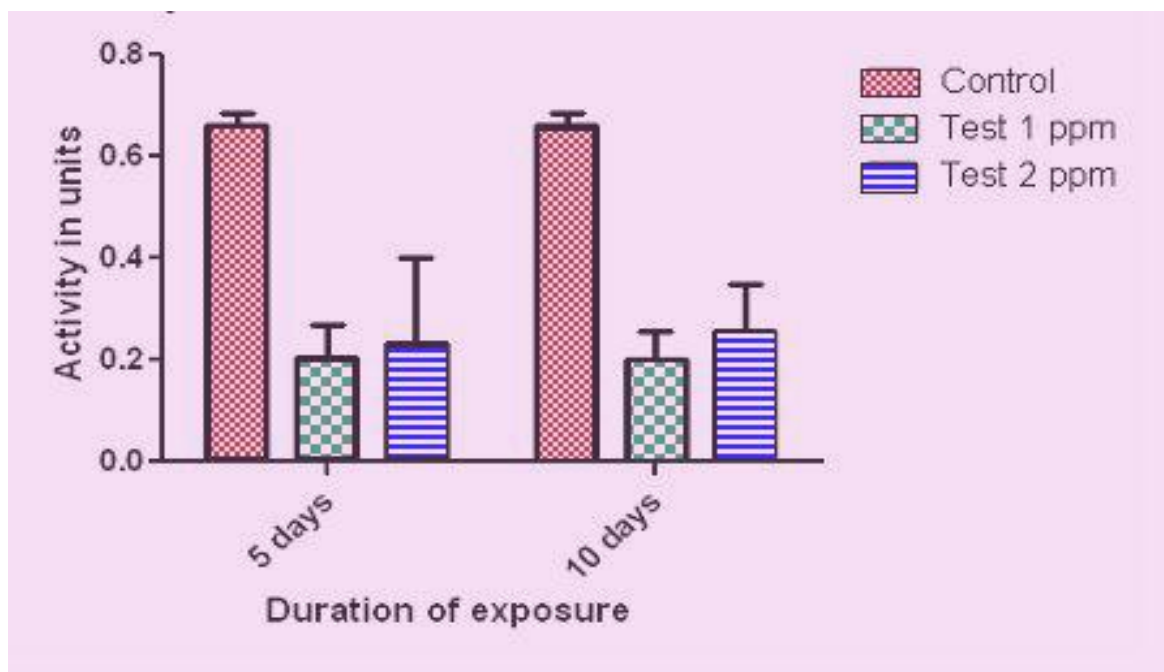


FIGURE NO 1: ACTIVITY OF ASPARTATE TRANSAMINASE IN THE LIVER OF EXPERIMENTAL FISHES

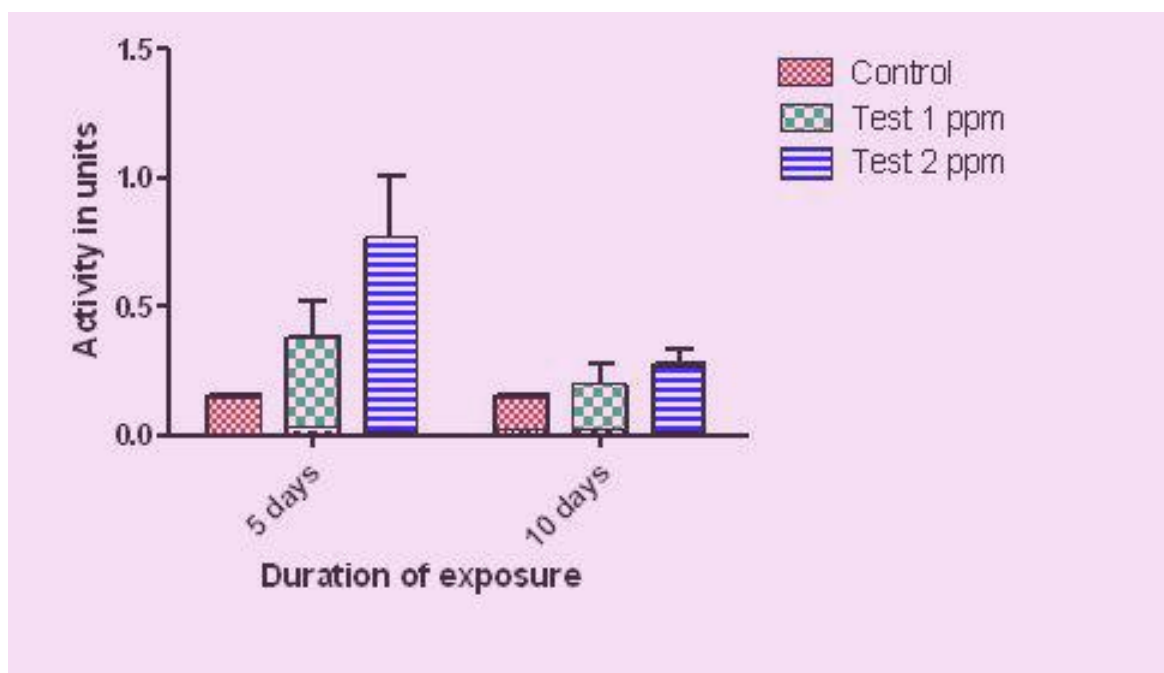


FIGURE NO 2: ACTIVITY OF ASPARTATE TRANSAMINASE IN THE MUSCLE OF EXPERIMENTAL FISHES

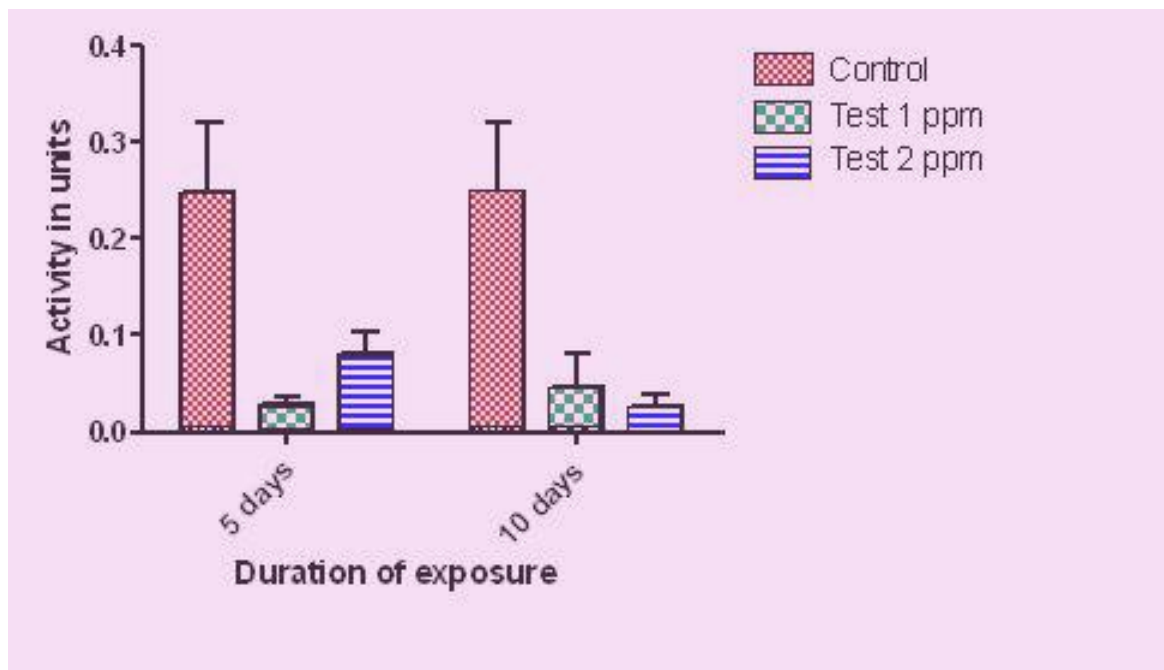


FIGURE NO 3: ACTIVITY OF ALANINE TRANSAMINASE IN THE LIVER OF EXPERIMENTAL FISHES

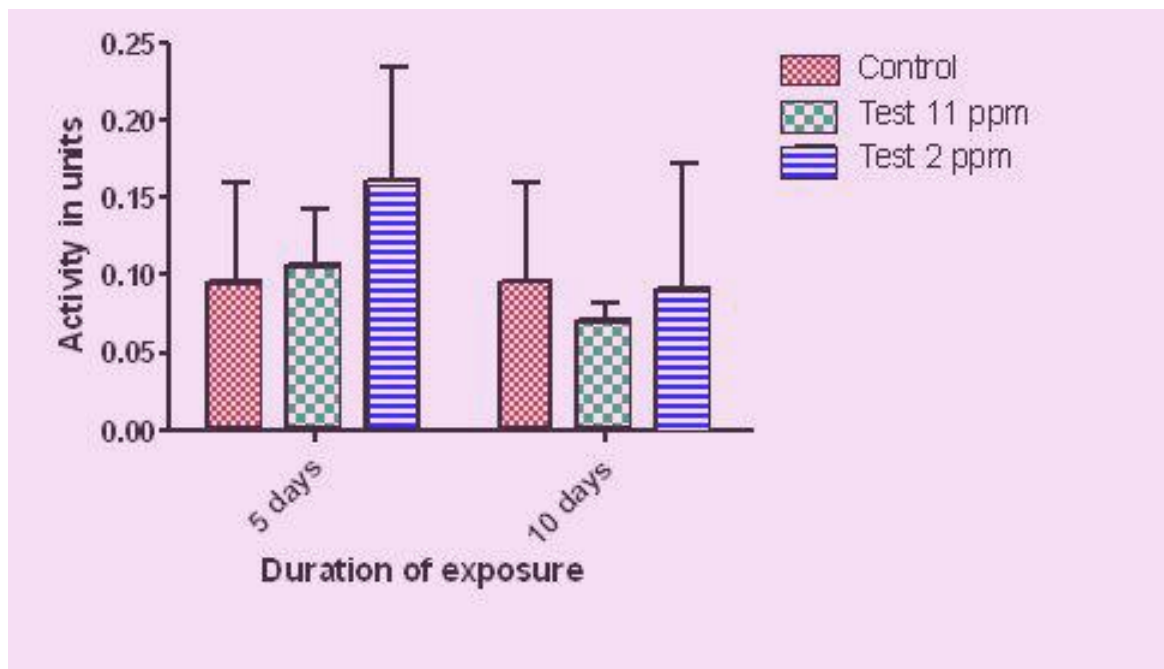


FIGURE NO 4: ACTIVITY OF ALANINE TRANSAMINASE IN THE MUSCLE OF EXPERIMENTAL FISHES

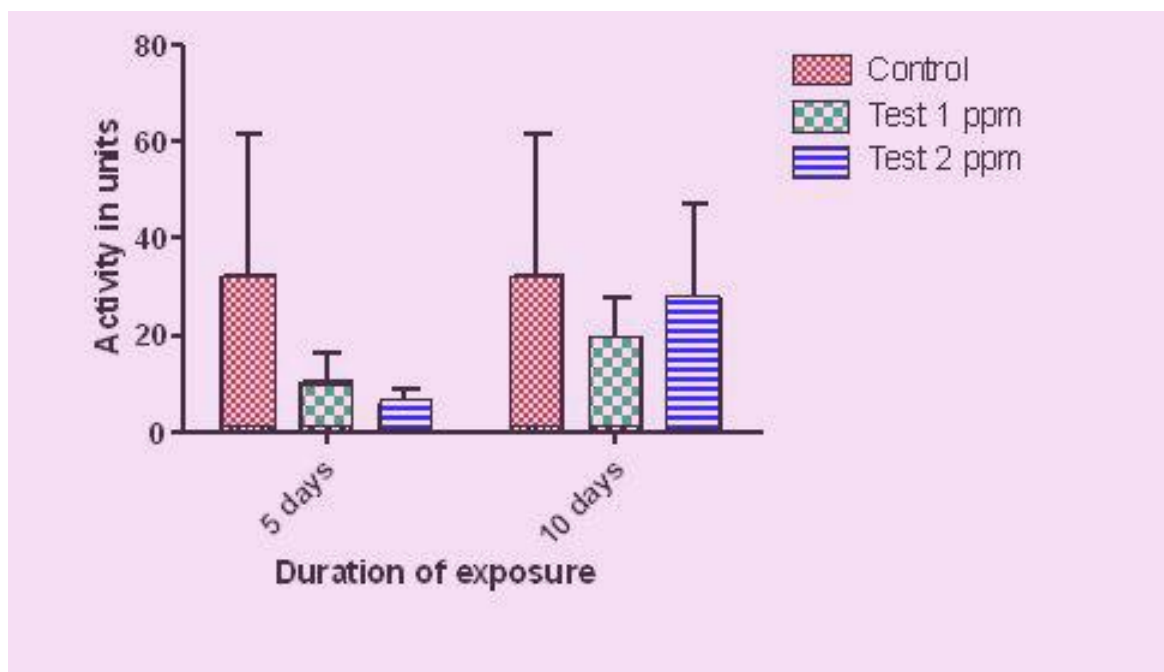


FIGURE NO 5: ACTIVITY OF CATALASE IN THE LIVER OF EXPERIMENTAL FISHES

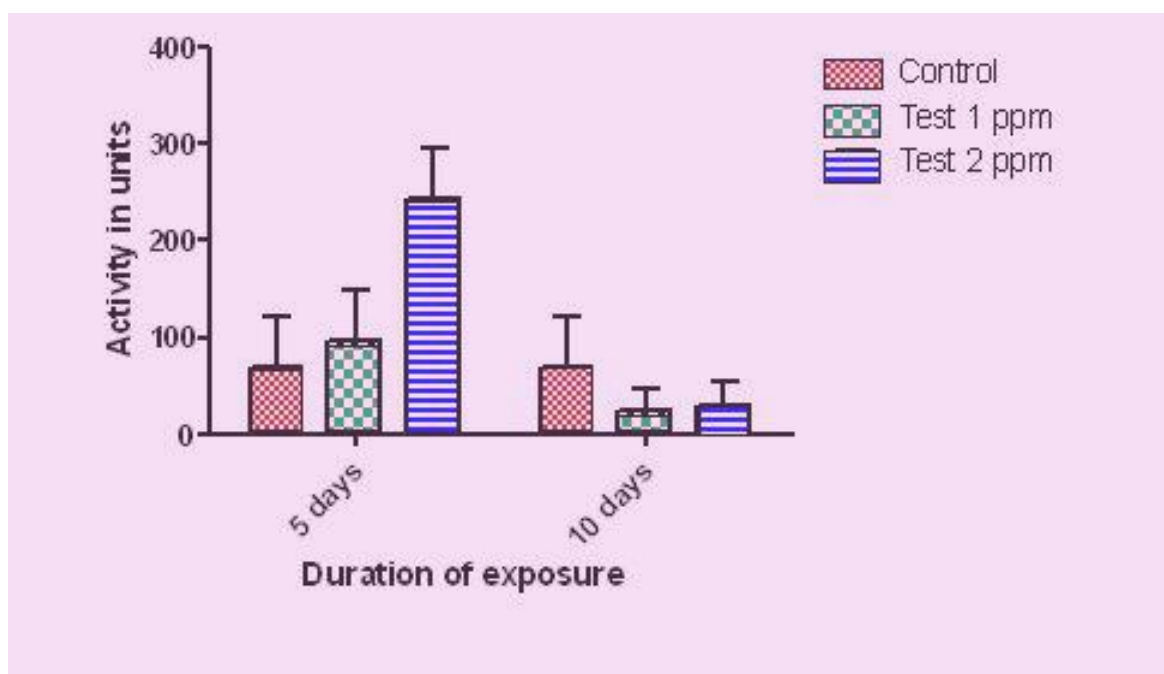


FIGURE NO 6: ACTIVITY OF CATALASE IN THE MUSCLE OF EXPERIMENTAL FISHES

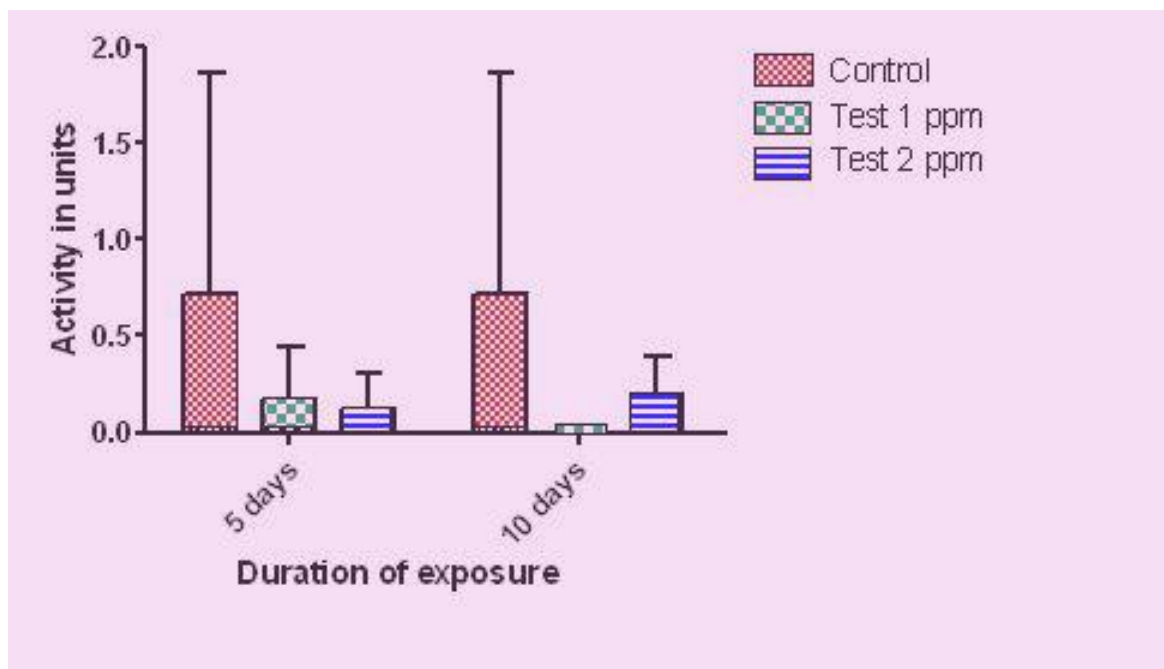


FIGURE NO 7: ACTIVITY OF SUPEROXIDE DISMUTASE IN THE LIVER OF EXPERIMENTAL FISHES

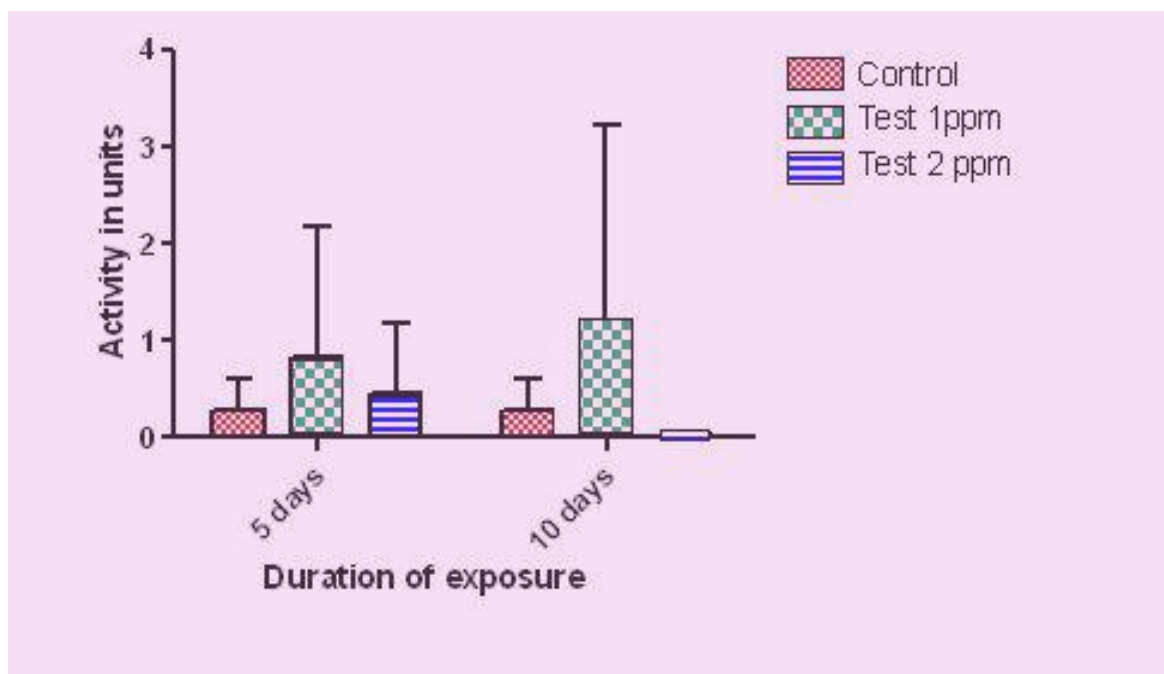


FIGURE NO 8: ACTIVITY OF SUPEROXIDE DISMUTASE IN THE MUSCLE OF EXPERIMENTAL FISHES

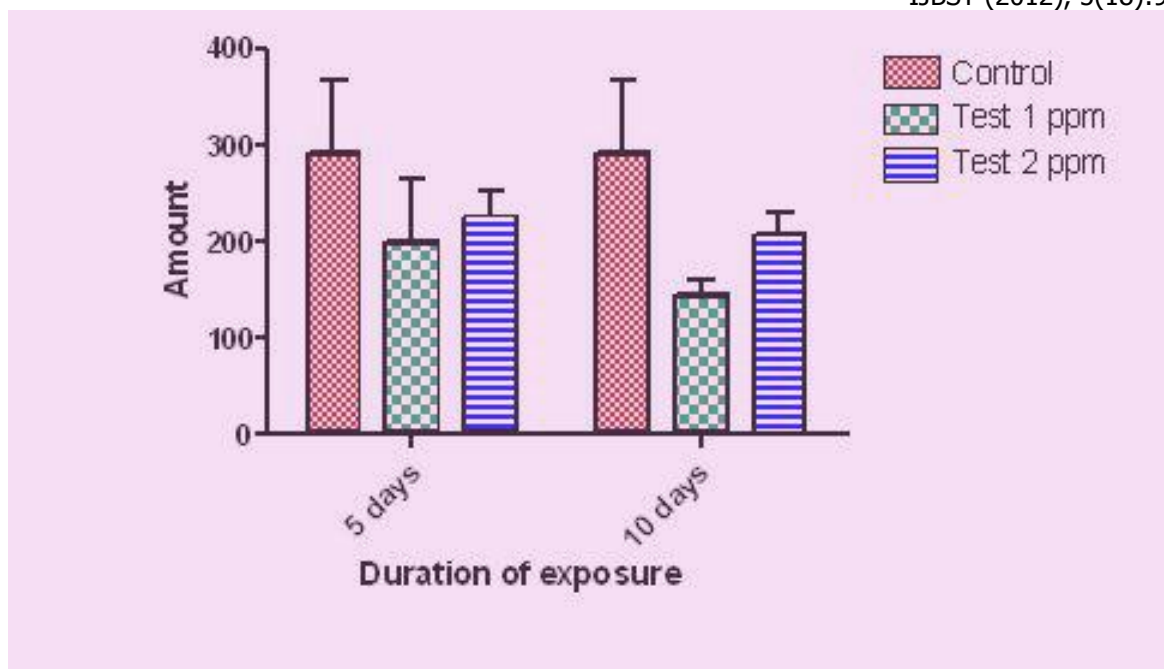


FIGURE NO 9: ACTIVITY OF REDUCED GLUTATHIONE IN THE LIVER OF EXPERIMENTAL FISHES

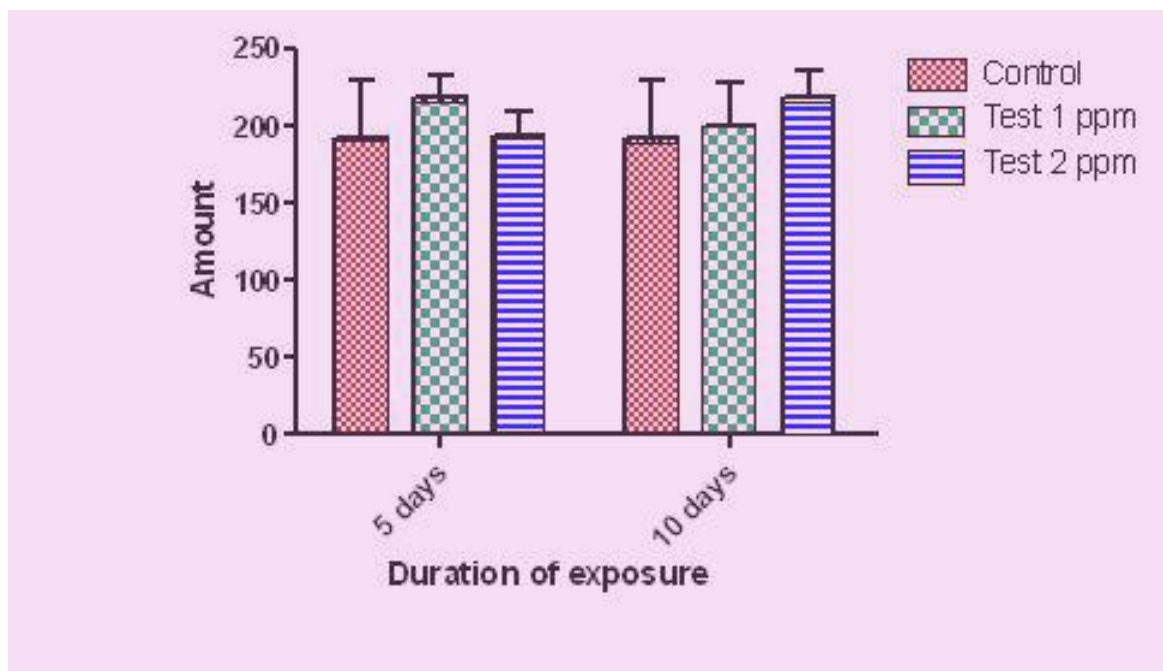


FIGURE NO 10: ACTIVITY OF REDUCED GLUTATHIONE IN THE MUSCLE OF EXPERIMENTAL FISHES

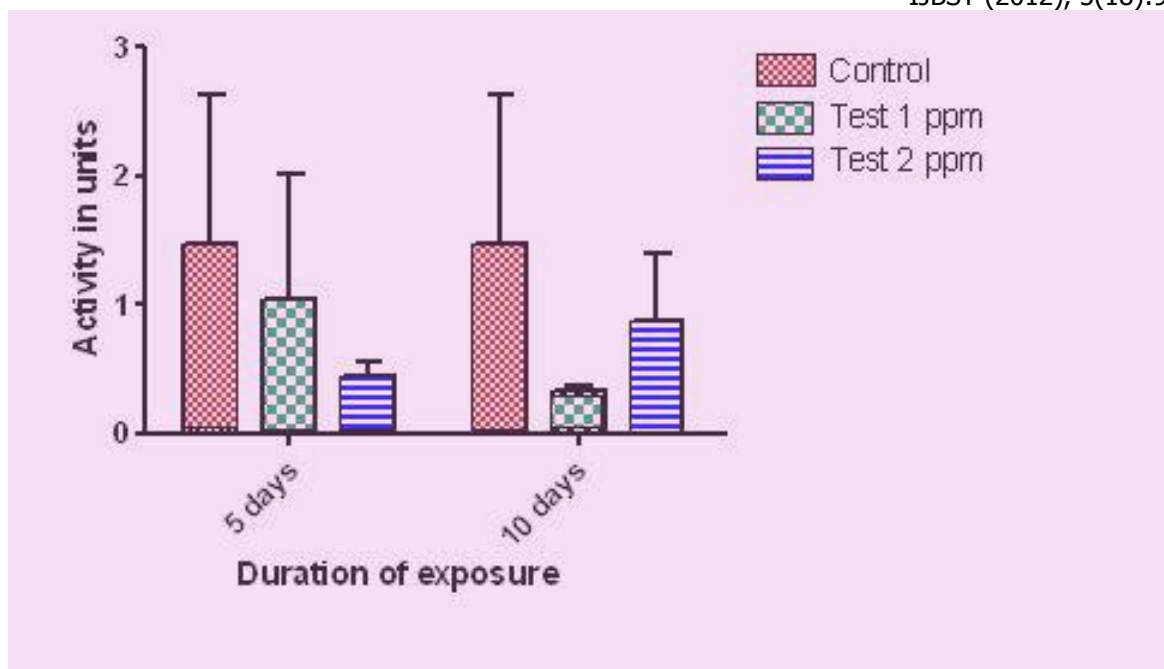


FIGURE NO 11: ACTIVITY OF GLUTATHIONE-S-TRANSFERASE IN THE LIVER OF EXPERIMENTAL FISHES

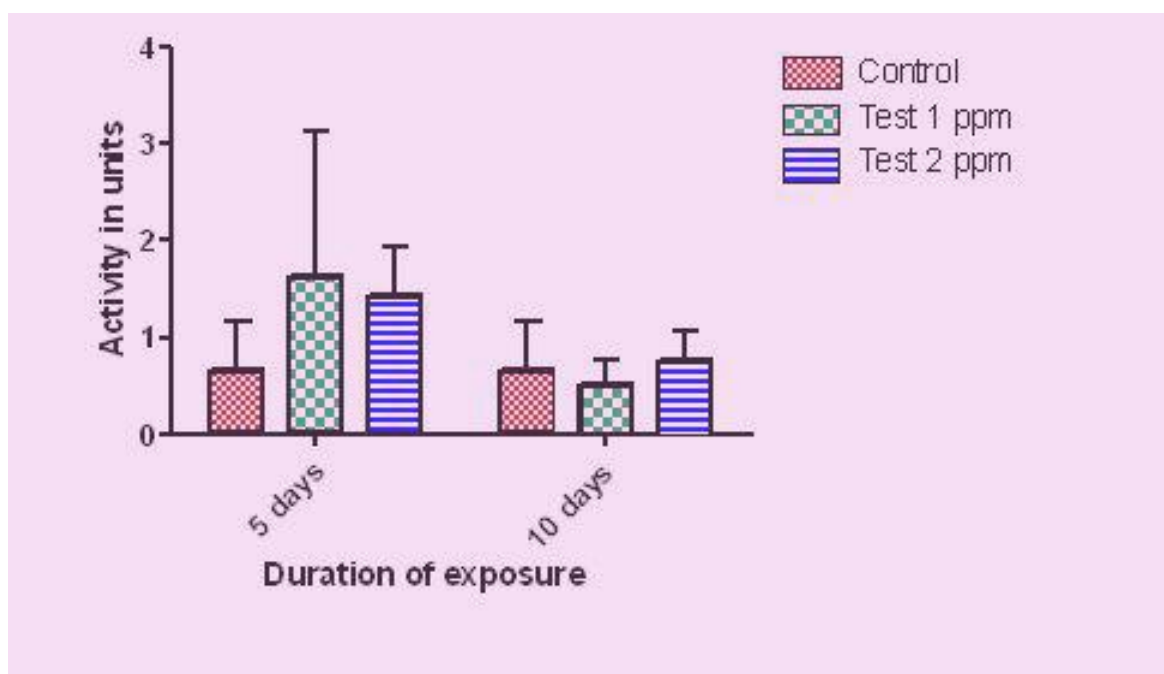


FIGURE NO 12: ACTIVITY OF GLUTATHIONE-S-TRANSFERASE IN THE MUSCLE OF EXPERIMENTAL FISHES

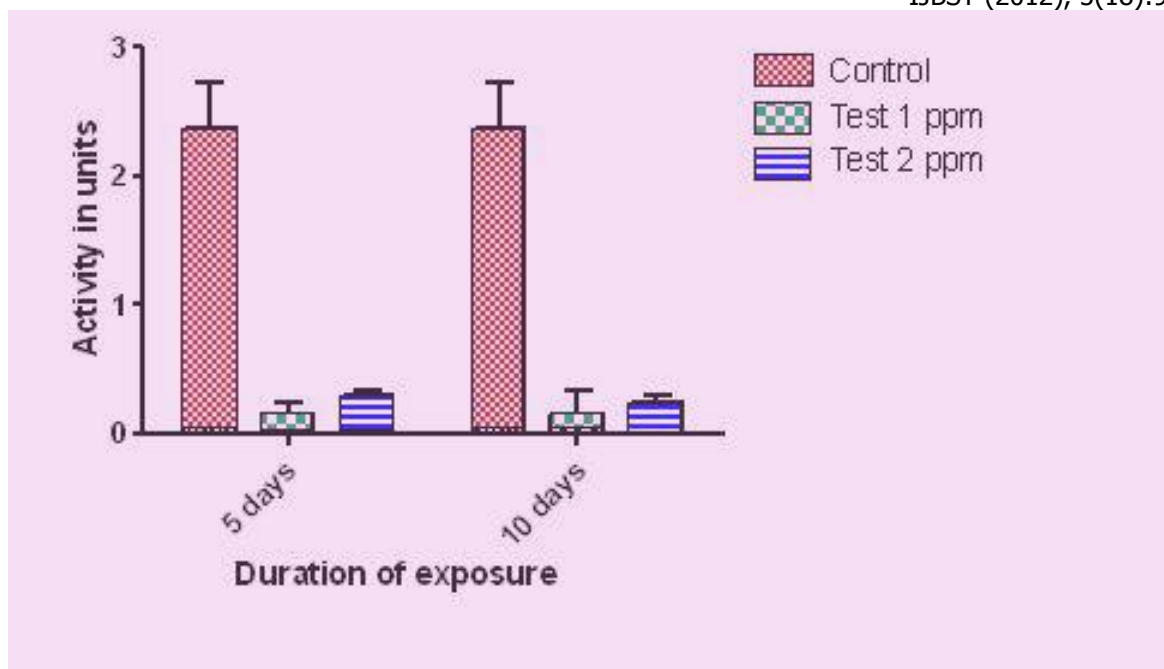


FIGURE NO 13: ACTIVITY OF GLUTATHIONE PEROXIDASE IN THE LIVER OF EXPERIMENTAL FISHES

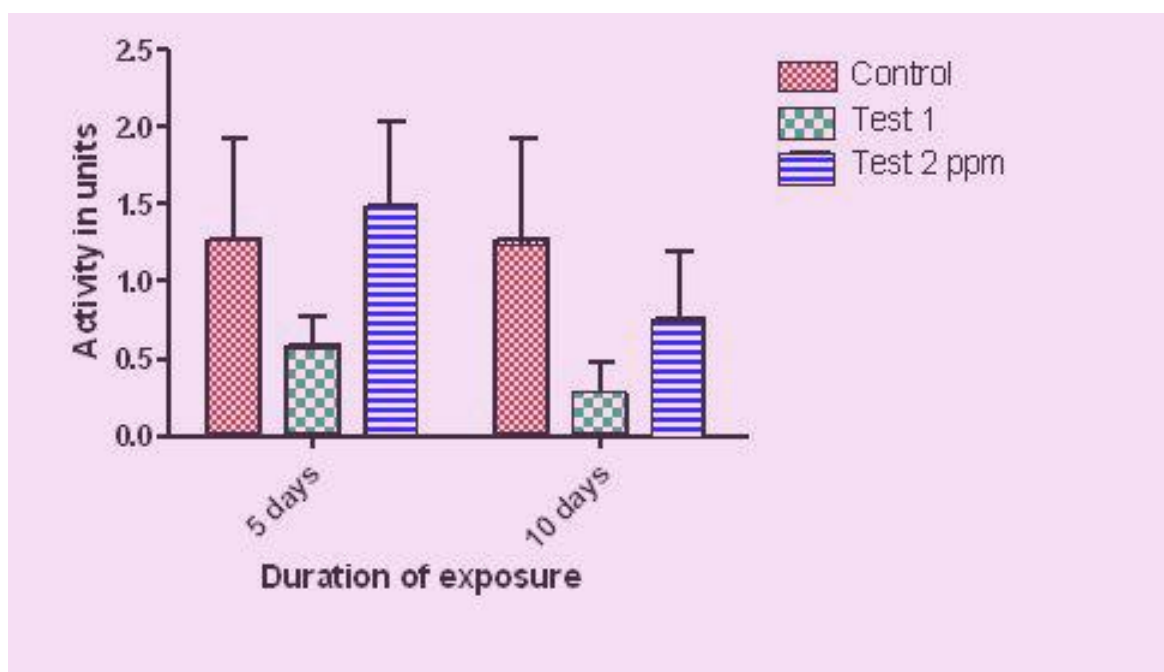


FIGURE NO 14: ACTIVITY OF GLUTATHIONE PEROXIDASE IN THE MUSCLE OF EXPERIMENTAL FISHES

CONCLUSION

The activity of Aspartate Transaminase, Alanine Transaminase, Catalase, Superoxide Dismutase, Reduced Glutathione, Glutathione –S-Transferase and Glutathione Peroxidase were highly significant in the liver of tannery treated fishes, compared to that of the control. There are chances of the transfer of toxic

heavy metals being accumulated in the fishes from the tannery effluent to the human beings through the food web. Hence, the effluents which are discharged into the water source should be effectively treated and then let out. This ensures safety to aquatic and human life.

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